

REMARKS

The fees for a three month extension of time should be charged to Deposit Account No. 02-1818. Any fees that may be due in connection with filing this paper or with this application during its entire pendency may be charged to Deposit Account No. 02-1818. If a Petition for extension of time is required, this paper is to be considered such Petition, and any fee charged to Deposit Account No. 02-1818.

Claims 1-6, 9, 12-16, 18 and 21-23 are pending. Claim 1 is amended to render it clear that bacteria are systemically administered, and then detected inside a subject to identify their location, which indicates the location of a wounded or inflamed tissue inside a subject. The claims thus render it clear that the claimed method is for detecting inflamed or wounded tissues inside a subject who has inflamed or wounded tissue (or does not), not a method for detecting or monitoring infection nor a method involving a step of burning a subject. Claim 23 is rewritten as an independent claim, incorporating all limitations of base claim 1 prior to amendment..

Claims 3-5, 13 and 15, which are directed to non-elected species, are withdrawn. They are retained pending allowance of a generic claim. Applicant reserves the right to file continuing/divisional applications to non-elected, cancelled and unclaimed subject matter.

REJECTION OF CLAIMS 1, 2, 6, 9, 12, 14, 16, 18 and 21-23 UNDER 35 U.S.C. §112, FIRST PARAGRAPH – SCOPE OF ENABLEMENT

Claims 1, 2, 6, 9, 12, 14, 16, 18 and 21-23 are rejected under 35 U.S.C. §112, first paragraph, because the Examiner alleges the specification, while enabling for a method for detecting a cutaneous wound comprising administering a bacterial cell selected from *E. coli* and attenuated *S. typhimurium* or attenuated *V. cholerae*, does not reasonably provide enablement for a method of detecting any wounded or inflamed tissue inside a subject. The Examiner also alleges that Example 2 shows that the accumulation of bacteria depends on the strain of bacteria used, the location of the wound, and the strain of mouse. The Examiner further alleges that Yu *et al.* demonstrates that bacteria, viruses or mammalian cells, when administered to subjects, accumulate in cancerous tissues, but not in any tissue recited in the claimed subject matter. This rejection respectfully is traversed.

Relevant law

To satisfy the enablement requirement of 35 U.S.C. §112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. *Atlas Powder Co. v. E.I. DuPont de Nemours*, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be met by providing sufficient disclosure, either through

illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything within the scope of a broad claim." *In re Anderson*, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of §112, first paragraph "can be fulfilled by the use of illustrative examples or by broad terminology." *In re Marzocchi et al.*, 469 USPQ 367 (CCPA 1971)(emphasis added).

Further, because "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." *In re Grimme, Keil and Schmitz*, 124 USPQ 449, 502 (CCPA 1960). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to broad claims that define the invention without a reference to specific instrumentalities. *Smith v. Snow*, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

Thus, there is no requirement for disclosure of every species within a genus. Applicant is entitled to claims are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed.

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the subject matter *as claimed*. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988).

Arguments

In setting forth the enablement rejection, the Examiner has alleged that the scope of the claims is not enabled in view of the factors enumerated in *In re Wands*. The Examiner has relied only on alleged unpredictability in the art with respect detection of wounded and inflamed tissues using any microorganism or cell. Predictability is only one of the nine or ten factors that must be considered and weighed. Further, the Examiner has provided no sound

scientific basis for such conclusion. As evidenced below, the state of the art, knowledge of those of skill in the art, the teachings in the application, and the working examples, as well as the demonstrated predictability and reproducibility of the methods, evidence that it would not require undue experimentation to practice the methods as claimed.

Consideration of the all factors enumerated in *In re Wands*, including the scope of the claims, the teachings and examples in the specification for administering and detecting bacteria for detection and treatment of wounded and inflamed tissue, the high level of skill of those in this art, the advanced knowledge of those of skill in the art, the fact that it is predictable given the teachings of the instant application and the state of the art at the time of the effective date of the claims, it would not require undue experimentation for one of skill in the art to practice the methods as claimed herein to introduce a detectable bacterium into a subject for the detection of a wounded or inflamed tissue within a subject. Furthermore, it does not require undue experimentation to select a bacterium with the properties as described in the application to practice the claimed methods. Specifically, one of skill in the art can select a bacterium that is detectable, non-pathogenic or attenuated, replication competent and that is recognized by the immune system of the subject to whom the bacterium is administered. As discussed, below in more detail, the specification, demonstrates that a bacterium with such properties predictably localizes to wounded and inflamed tissues, and can be detected. Further, since the bacterium accumulates in wounded and inflamed tissues it delivers encoded proteins thereto, including proteins expressed for therapy of wounded and inflamed tissues.

As established below, the instant claims are directed to methods that employ bacteria that are detectable and that can be administered to subjects. As shown in the application that bacteria that are non-pathogenic and recognized by the immune system accumulate in any wounded or inflamed tissues. The bacteria include are modified to include a detectable product or to encode products and substrates to produce or induce a detectable signal. Such detectable products and signals are well known. Methods for detecting such bacteria in subjects are known and also are described in the application.

Bacteria that can be administered and that are non-pathogenic and recognized by the immune system are known, and exemplified and described in the application. Such bacteria, not only are known and exemplified, they also can be prepared, as taught in the application, for detection of wounded and inflamed tissues. The instant application provides a new use and method using known materials and detection/visualization methods. The use of bacteria and their accumulation in tumor tissues is known in the art. The instant application shows

that such bacteria also accumulate in wounded and inflamed tissue inside of a subject and that known detection/visualization methods can be employed to detect accumulation of the bacteria and thereby detect wounded and inflamed tissues.

As recited in the claims, the bacteria are not *any* bacteria, but are bacteria that are (a) detectable; (b) able to replicate in the subject to whom the bacteria are administered; (c) non-pathogenic or attenuated; and (c) recognized by the immune system of the subject. The specification teaches numerous species of bacteria suitable for practice of the method and how to identify other species, the specification includes several working examples, with a variety of bacterial species, and teaches of the properties of bacteria for use in the methods. Those of skill in the art, who have a high level of skill, know of such bacteria and how to employ them for administration and visualization. Visualization/detection methods are known to those of skill in the art and also are described in the specification. Further, the specification demonstrates that the method is reproducible (*i.e.*, predictable). Thus, based on these factors, the factors enumerated in *In re Wands*, which include the scope of the claims, the teachings and examples in the specification, level of skill in the art, knowledge of those of skill in the art and state of the prior art, and predictability, it would not require undue experimentation for one of skill in the art to practice the methods as claimed to introduce detectable bacteria into a subject for the detection of a wound, wounded tissue, inflammation or inflamed tissue.

1. Breadth of the claims

Claim 1 recites:

A method for detecting wounded or inflamed tissue inside of a subject, comprising:
identifying a subject to be tested for the presence or absence of wounded tissue or inflamed tissue therein;
systemically administering to the subject in whom the presence or absence of a wounded tissue or inflamed tissue is to be detected, a bacterium, wherein:
the bacterium is detectable in the subject;
the bacterium replicates in the subject;
the bacterium is not pathogenic to the subject and is recognized by the immune system of the subject;
the bacterium is not targeted; and
after a sufficient time for the bacterium to accumulate in wounded or inflamed tissues inside of the subject, imaging the detectable bacterium to detect accumulation of the bacterium in the subject, thereby detecting or imaging the wounded or inflamed tissues inside of the subject.

Claim 2 is dependent on claim 1 and recites that the bacterium encodes a protein(s) for the therapy of the detected wounded or inflamed tissue. Other dependent claims specify

therapeutic proteins, detectable proteins or proteins that induce a detectable signal and detection methods.

As noted, Applicant reserves the right to prosecute cancelled subject matter, such as that to viruses, in continuing/divisional applications. As pending, the claims are tailored to the teachings and working examples in the specification. As discussed below, bacteria that meet the recited criteria (replicates in the subject, are not pathogenic to the subject, and are recognized by the immune system of the subject) are well known and numerous examples are taught and exemplified in the specification. Further detectable bacteria or bacteria modified for detection also are taught and exemplified in the specification and also are well known to those of skill in the art. Thus, the claims are of the same scope as the specification

2. Level of Skill in the Art

The level of skill in this art is recognized to be high (see, *e.g.*, *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). The numerous articles and patents made of record in this application, authored and reviewed by those known in the art, further evidences the high level of skill in this art.

3. State of the Prior Art

At the time of filing of the application, a broad body of knowledge had amassed in the areas of microbiology, molecular biology, genetics, and medicine including many technical procedures covering the generation, preparation, administration and detection of bacteria, viruses, and cells, including production of recombinant organisms using recombinant nucleic acid techniques, and expression and detection of exemplary detectable proteins, which are employed in the claimed methods. Numerous such procedures are referenced in the specification and/or described in the application and/or described in prior art submitted by Applicant to the Patent Office in connection with the instant Application.

As described in the application, the criteria for bacteria to be used in the method are: attenuated/non-pathogenic and the ability to replicate in the subject. The art provides numerous species of microorganisms that meet these criteria and that can be used in the methods provided in the instant application. For example, Pawelek *et al.* (WO 96/40238) provides numerous examples of attenuated or non-pathogenic bacteria and other microorganisms that can be administered to mammalian subjects (see Table 1 of WO 96/40238, which lists many species of bacteria for administration). The reference also provides techniques for attenuation of the bacteria and methods for engineering the bacteria to express heterologous genes. Also provided are methods for selection of suitable bacteria for administration.

Numerous detectable proteins were known in art at the time of filing that can be used in the claimed methods. Exemplary detectable proteins include luminescent or fluorescent proteins, such as luciferase from *Vibrio harveyi* (Belas *et al.*, *Science* 218 (1982), 791-793) and from *Vibrio fischerii* (Foran *et al.*, *Nucleic Acids Res.* 16 (1988), 177), firefly luciferase (de Wet *et al.*, *Mol. Cell. Biol.* 7 (1987), 725-737), aequorin from *Aequorea victoria* (Prasher *et al.*, *Biochem.* 26 (1987), 1326-1332), Renilla luciferase from *Renilla reniformis* (Lorenz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88 (1991), 4438-4442) and green fluorescent protein from *Aequorea victoria* (Prasher *et al.*, *Gene* 111 (1987), 229-233). Furthermore, expression of proteins that can be employed for detection via magnetic resonance or positron emission imaging were known (see, *e.g.*, WO 01/25399, Weissleder *et al.* (2000) *Nature Medicine* 6(3): 351-354, Weissleder *et al.* (1992) *Magnetic Resonance Quarterly* 8(1): 53-63, Tjuvajev *et al.* (2001) *Journal of Controlled Release* 74(103): 313-315, Moore *et al.* (1998) *Biochimica et Biophysica Acta* 1402(3): 239-249).

Techniques for construction light-emitting bacteria were known at the time for filing of the instant application. For example, bacteria engineered to carry the *lux-cdabe* operon for expression of bacterial luciferase and administration methods for infecting mice with such bacteria were well-known. Meighen *et al.*, *J. Bacteriol.* 174 (1992), 5371-5381 and Lee *et al.*, *Eur. J. Biochem.* 201 (1991) 161-167, Fernandez-Pinas *et al.*, *Gene* 150 (1994), 169-174, describe the *lux* operon and construction of a wide variety of bacteria that contain the operon for expression of the bacterial luciferase.

Expression of detectable proteins by microorganisms for detection of microorganisms within other organisms are described in the art and include, for example, expression of *luxAB* in *Rhizobia* residing within the cytoplasm of cells of infected root nodules (Legocki *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 83 (1986), 9080-9084; O'Kane *et al.*, *J. Plant Mol. Biol.* 10 (1988), 387-399), *Bacillus subtilis* and *Bacillus megatherium* expression of *lux A* and *lux B* fusion genes (Fab2) in insect larvae and worms (Escher *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 86 (1989), 6528-6532), and *Pseudomonas* or *Ervinia spp.* expression of pathogen-activated PAL promoter-bacterial luciferase fusion gene in transgenic Arabidopsis plants, tomato plants and stacks of potatoes (Giacomin *et al.*, *Plant Sci.* 116 (1996), 59-72). Expression of light-emitting bacteria in mammalian subjects is described in Contag *et al.*, *Mol. Microbiol.* 18 (1995), 593-603.

Methods for detection of microorganisms, such as bacteria, that express light-emitting molecules also were known at the time of the earliest priority date. International PCT application No WO01/14579, which describes the use of targeting bacteria to tumor antigens

for detection of tumors, describes many non-pathogenic bacteria that replicate in subjects, as well as methods for detection thereof. Numerous other references describe bacteria and methods for detection. For example, luminescent and fluorescent signals produced by such proteins can be detected with low light imaging cameras or fluorescent imaging devices (see, Engebrecht *et al.*, *Science* 227 (1985), 1345-1347; Legocki *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 83 (1986), 9080-9084; Chalfie *et al.*, *Science* 263 (1994), 802-805).

Contag *et al.* (U.S. Patent No. 6,217,847) describes methods of *in vivo* imaging, including bacteria and viruses that express detectable proteins. Contag *et al.*, which requires targeting of bacteria, not accumulation, provides numerous examples of light-emitting proteins that can be expressed by the bacteria (see, *e.g.* columns 9-10) and methods for detecting the bacteria including several types of photodetection and amplification devices (see, *e.g.* columns 16-17). In addition, the reference also provides a detailed description of the methods that can be employed to image the bacteria *in vivo* (see, *e.g.* columns 17-20).

Zhao *et al.* (2001) *Proc. Natl. Acad. Sci. U.S.A.* 98(17) 9814-9818 describes methods of engineering GFP-expressing *E. coli* for *in vivo* detection. Methods for preparing, administering and detecting the bacteria *in vivo* are provided. The reference describes a method of tracking the fluorescent signal emitted by the bacterium in a live animal over time. The reference thus describes methods of spatial and temporal imaging of bacterial localization within a subject.

In addition, techniques and methods for expression and *in vivo* detection of fluorescent and bioluminescent molecules are known (see, *e.g.*, Belas *et al.*, *Science*, 218: 791-793 (1982), Chalfie *et al.*, *Science* 263: 802-805 (1994), Contag *et al.*, *Mol. Microbiol.* 18: 593-603 (1995), Greer III, *et al.*, *Luminescence*. 17(1):43-74 (2002), Rodriguez *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* U.S.A., 85: 1667-1671 (1988), Rocchetta *et al.*, *Antimicrobial Agents and Chemotherapy* 45(1): 129-137 (2001), Yang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 97(22): 12278-12282 (2000), Wang Y. *et al.*, *Mol Genet Genomics*. 268(2):160-8 (2002), Lamberton *et al.*, *Proceedings of the 12th International Symposium on Bioluminescence & Chemiluminescence*: 5-9 April 2002, Robinson College, University of Cambridge, UK, p 3.22 (2002)).

Techniques and methods for use of other detectable molecules for *in vivo* labeling such as radionucleotides for MRI or PET imaging are known (see, *e.g.*, Welling *et al.*, *Eur J Nucl Med*. 27(3):292-301 (2000), Adonai *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 99: 3030-3035 (2002), Welling *et al.*, *Nucl Med Biol*. 29(4):413-22 (2002), Nibbering *et al.*, *Nucl Med Commun*. 19(12):1117-21 (1998), Weissleder, T. *et al.*, *Nat. Med.*, 6(3): 351-354 (2000) and

Berger, F. and S.S. Gambhir, *Breast Cancer Research* 3: 28-35 (2001); other references describing imaging methods, include, *e.g.*, references cited in Massoud *et al. Genes & Development* 17: 545-580 (2003)). Engineering bacteria to accumulate metals and metal-binding proteins were well-known before the earliest priority date of the instant application.

The references cited above are not an exhaustive list of the references that were available to one of skill in the art at the time filing. They are a representative selection of art to demonstrate the existence large volume of information regarding tested and reliable procedures available at the time of filing of the instant application and thus evidence the advanced state of the art at the relevant time with regard to known methods of methods of selection of, modifying, administering and detecting microorganisms and cells, including bacteria and viruses. They evidence the advanced state of the art with respect to identification of bacteria with properties recited in the claims, and methods for detection/visualization.

4. Nature of the Claimed Subject Matter

The claimed subject matter is a method for detecting wounded and inflamed tissues/sites by administration of bacteria that are detectable, non-pathogenic, replication competent and recognized by the immune system. The application teaches and demonstrates and exemplifies that such bacteria accumulate in wounded/inflamed tissues/sites. As discussed above, the claims are directed to methods of use of known materials (bacteria) that can be detected/visualized by known methods. Hence, the reagents used in the methods and the detection methods should not be at issue. Once Applicant describes the use of such reagents for detection of wounds/inflamed tissues/sites and describes bacteria and properties thereof more should not be needed for one of skill in the art to practice the methods as claimed.

5. Predictability of the method

The specification provides three working examples with three different species of bacteria, and shows that all, as described in the specification accumulate wounded and inflamed tissues/sites. The data show that an animal with a wounded or inflamed tissue can be administered an attenuated, non-pathogenic bacterium that is recognized by the immune system and the administered bacteria accumulates in the wounds or inflamed tissues, thereby allowing detection of the wound.

Practice of the method with other bacteria in addition to the exemplified species and detection methods is routine, since the claims and specification recite the properties of the bacteria required, including that they are non-pathogenic and recognized by the immune

system, and the specification describes such bacteria. As described above, a variety of non-pathogenic or attenuated bacteria recognized by the immune system were known at the time the priority date of the application, as were methods for detection thereof for visualization of tissues/sites as described above (see references discussed above, and the specification as discussed herein). Hence there is no basis to conclude that successful practice of the method is not predictable.

6. Amount of Direction and Guidance Provided by the Specification

The specification describes the generation, administration, and detection of microorganisms and cells, including bacteria, for the detection of a wound, wounded tissue, an inflammation site, inflamed tissue, or a disease or condition associated thereof. The teachings of the specification describe how to select bacteria or use in the methods, how to administer them, how to detect them *in vivo*, and provides instruction for modification of bacteria to express proteins, including therapeutic proteins and detectable proteins. The specification teaches the features of the bacteria for use in the methods (*i.e.*, that they are, non-pathogenic or attenuated and recognized by the immune system). It is taught that such detection is useful for visualization of and diagnosis of the wounded or inflamed tissues and for therapy of the wounded or inflamed tissues, including identification of site for subsequent application of a therapeutic agent (page 5, line 25 through page 6, line 6) or directed expression of proteins suitable for therapy at the affected site.

The specification teaches examples of proteins that can be expressed by the microorganism or cell for diagnosis and treatment (page 6, line 8). For example, diagnostic proteins, such as fluorescent, bioluminescent, and metal binding proteins (see, *e.g.*, pages 7, line 13-18, pages 8-10) and therapeutic proteins, such as various growth factors and enzymes (see, *e.g.*, pages 6-7) can be expressed by the microorganisms or cells and are described. Exemplary vectors including viral, mammalian and bacterial vectors for the expression of such proteins are also exemplified (see, *e.g.*, page 8).

The specification teaches exemplary bacteria, such as attenuated *Salmonella typhimurium*, attenuated *Vibrio cholera*, attenuated *Listeria monocytogenes* and *E. coli* and shows that these species accumulate in wounded/inflamed tissues. The specification also teaches the properties of the bacteria that result in accumulation –*i.e.*, that the bacteria replicate in the subject, are recognized by the immune system of the subject and are non-pathogenic or attenuated.

The specification also teaches the property and reason why the bacteria that are systemically administered accumulate in the wounded/inflamed. The specification teaches

that the wounded/inflamed tissues are “immunoprivileged,” which the specification describes as sheltered from the immune system of the subject. Thus, the specification teaches that bacterium, which are non-pathogenic/attenuated and recognized by the immune system are cleared from the subject, except in immunoprivileged tissues. The instant application teaches that that wounded/inflamed tissue inside of a subject is immunoprivileged. Thus, bacteria with the requisite properties, will, upon systemic administration accumulate in such tissues.

The specification further teaches methods of administering the bacteria, including routes of administration and factors to be considered for assessing methods of administration and dosages (see, *e.g.* Examples). The specification also provides examples of diseases and conditions that are associated with wounded or inflamed tissue. The specification also provides details for administration of therapeutic proteins and molecules (see, *e.g.*, pages 14-15) with the bacteria.

7. Working Examples

The specification provides working examples and descriptions of the generation, administration, and detection of microorganisms and cells for the detection of a wound, wounded tissue, an inflammation site or inflamed tissue. The working examples of bacterial accumulation at wounded or inflamed sites provide sufficient teachings, in combination with what was known to those of skill in the art at the time of the instant application’s earliest priority date, to generate, administer, and detect a microorganism or cell regardless of the microorganism or cell that is used provided that the microorganism or cell is detectable. For example, techniques for use in the administration and detection of luminescent bacteria in an animal model for wounded tissues are provided in Examples 1, 2 and 3, including examples of plasmid constructs that can be used for bacterial expression of bacterial luciferase, administration methods (*e.g.* intravenous injection), methods and equipment employed for detection, and methods for generating wounded tissue for the experiment, including incision wounds, ear tags wounds, and surgical heart defects. Such techniques are applicable to use of the methods in subjects with existing wounds or inflamed tissues. Furthermore, the examples provide guidance for one of skill in the art, if needed, to use animal models for testing particular detectable microorganisms and cells. For example, methods and instruction are provided for analysis of accumulation in wounded/inflamed tissue versus unaffected tissue in an animal model, including whole body luminescence detection as well as organ excision and cell culture methods for analyzing various tissues of the animal model.

The working examples exemplify the teaching using three diverse species of bacteria (*S. typhimurium*, *V. cholera*, and *E. coli*). Each of these bacteria were modified to express a

protein that induces a detectable signal, a bacterial luciferase, which is expressed from the *lux-cdabe* operon. Also expressed from the *lux-cdabe* operon are proteins involved in the production of the substrate for the luciferase, which allows detection of the bacteria. The examples demonstrate exemplary methods of systemic administration of the microorganisms by intravenous administration. Following intravenous administration, the bacteria are initially carried throughout the body via the blood stream as shown in Figure 1. After a period of time, *S. typhimurium* and *V. cholera* were shown to accumulate in cutaneous wounds and inflamed tissues of the ear (Example 2). In Example 3, *E. coli* was shown accumulate **in wounded heart tissue**. In all cases, the bacteria was efficiently cleared from non-wounded tissues by either the subject's immune system or organs normally involved in bacterial clearance, such as the liver and spleen. Given the normal function of the liver and spleen in bacterial clearance, initial accumulation of bacteria in the liver and spleen was observed in some instances.

The specification teaches that these are exemplary and that the examples can be extrapolated and use for any species of bacteria that exhibits the requisite properties of replicating in subject, being recognized by the immune system of the subject, and being attenuated/non-pathogenic. See, *e.g.*, pages 7 of the application, which recites:

Any microorganism or cell is useful for the diagnostic and therapeutic uses of the present invention, provided that it replicates in the organism, is not pathogenic for the organism *e. g.* attenuated and, is recognized by the immune system of the organism, etc. The terms "microorganism" and "cell" as used herein refer to microorganisms and cells which are per se not targeted to wounded or inflamed tissues (*i. e.* they cannot differentiate between wounded or inflamed tissues and the non-wounded or non-inflamed counterpart tissues) since the results of the experiments leading to the present invention show that microorganisms and cells accumulate in wounded or inflamed tissues due to the fact that in this environment they are not exposed to attack by the immune system of the host.

Applicant, thus, describes the requisite properties of suitable bacteria and, exemplifies several species. Applicant is not required to provide data or illustrative examples in support of every embodiment within the scope of a claim. *In re Anderson*, 176 USPQ 331, at 333 (CCPA 1973)).

8. Quantity of Experimentation

The type and quantity of experimentation in light of the teachings of the specification and knowledge and skill of those in the art is routine. As discussed above, the specification teaches the properties of bacteria for use in the methods and exemplifies the methods with three different species. Those of skill in the art are familiar with other suitable species, as well as detection/visualization methods. Accordingly, any experimentation with different

species would be routine to confirm, if necessary, that a particular species is accumulates in wounded/inflamed tissues, and optimization of parameters for such species. The specification teaches and exemplifies how to test species and parameters to optimize.

As evidenced by the state of the art discussed above, any needed manipulations to prepare bacteria have been practiced for many years. Furthermore, detection techniques for *in vivo* detection and methods to optimize such techniques are also well known. The application also provides ample guidance for routine testing of a microorganism or cell for use in diagnosis or treatment of wounded or inflamed tissues. "A considerable amount of experimentation is permissible, if it is merely routine..." *In re Wands* 858 F.3d 731, 737.

Conclusion

It respectfully is submitted, that although use of bacteria that are recognized by the immune system and replicate in the subject for detection of wounded or inflamed tissues was not known as of the earliest claimed priority date of the subject application (as this is the presently claimed subject matter), the specification provides working example demonstrating three different species of bacteria and showing that each accumulates in wounded/inflamed tissue, and the specification teaches the requisite properties of bacteria for use in the methods. Further, species of bacteria that at are recognized by the immune system and that are nonpathogenic or attenuated that can be used in the are known to those of skill in the art, and hence, in view of the teachings in the specification, one of skill in the art, routinely can select bacterial species to practice the methods. It is emphasized that the bacteria for use in the claimed methods are not any bacteria, as alleged in the Office Action, but rather, bacteria that are detectable, replication competent, non-pathogenic or attenuated and recognized by the immune system. Selection of bacteria with these properties is known in the art and taught in the application. Furthermore, use of such bacteria in the methods as claimed to detect wounded and inflamed tissues is reproducible and based on known methods and reagents, and this is predictable. The level of skill in the art is high, and knowledge of those of skill in the art is extensive, and there is a body of prior art that describes preparation of the reagents and use of the detection methods required for practice of the claimed methods. Thus, it would not require undue experimentation to practice the methods as claimed.

Applicant is entitled to claims that are commensurate in scope not only with what Applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the Applicant has disclosed. In the instant application, Applicant discloses to the public methods for the detection of wounded or inflamed tissue using known bacterial species that are detectable and known for detection by

known methods. Among Applicant's contributions, is the new use for the non-targeted bacteria for detection wounded/inflamed tissues or sites. The specification clearly shows that bacteria with the properties recited in the claims accumulate at such sites and can be detected at such sites.

Therefore, in view of the breadth of the claims, the high level of skill of those in the art, the knowledge of those of skill in the art, the teachings in the prior art, the teachings in the application, the working examples in the application, and the demonstrated repeatability of the method as claimed, it would not require undue experimentation to practice the methods as claimed. Thus, the claims are not broader than the enabling disclosure.

REBUTTAL TO THE EXAMINER'S ARGUMENTS

a. The Examiner states:

Nature of the invention and Breadth of the claims: The instant claims are directed to a method comprising administering a bacterium that is detectable in a subject and monitoring the subject to detect accumulation of the bacterium at wounded tissue or inflamed tissue inside the subject, whereby detection of the accumulation indicates the location of the wounded tissue or inflamed tissue. The specification states, "Any microorganism or cell is useful for the diagnostic and therapeutic uses of the present invention, provided that it replicates in the organism, is not pathogenic for the organism e.g. attenuated and, is recognized by the immune system of the organism, etc." (Paragraph bridging pages 6-7.) Thus, the claims broadly encompass practicing the claimed method using any bacterium having the capacity to accumulate at the location of any wound or any site of inflammation with sufficient specificity that the detectable accumulation of the microorganism can be used to indicate the location of a wounded or inflamed tissue.

As discussed above, the claims encompass the use of any bacterium that possesses the three listed properties: "the bacterium replicates in the subject; the bacterium is not pathogenic to the subject and is recognized by the immune system of the subject." Possession of these three properties is sufficient to ensure accumulation in wounded/inflamed tissue in the subject because, as taught in the application, wounded/inflamed tissue is immunoprivileged. NO "specificity" or targeting of the bacteria is required.

b. The Examiner continues:

Amount of direction provided by the inventor and existence of working examples: In support of the claimed invention, Applicant discloses that luminescent *S. typhimurim* and *V. cholera* injected into the femoral vein of nude mice and C57BU6J mice specifically accumulated at the site of cutaneous wounds. (Example 2, Figures 2-4). Given applicants arguments against certain prior art references, it is doubtful whether or not these can be considered working examples of the instantly claimed subject matter, as cutaneous wounds (such as those found in Hamblin et al) according to applicants are not to be considered "inside" a subject). It is further noted that the data presented (which consists of a single mouse for each condition) appear to show that the accumulation of bacteria depends on the strain of bacteria used, the location of the wound and the strain of mouse. For example, the nude mouse injected with Salmonella exhibits accumulation of bacteria at the leg wound

and ear tag (Figure 2B), the nude mouse injected with *Vibrio* exhibits accumulation only at the leg wound (Figure 3B), while the immunocompetent mouse exhibits accumulation of *Vibrio* only at the ear tag (Figure 4). Thus, the working examples demonstrate that the accumulation of any given strain of bacteria at any given wound in any given animal is highly variable and unpredictable.

Applicant respectfully disagrees. The data and examples are model systems of internal wounds/inflamed tissues. The bacteria are systemically (intravenously) administered and shown to accumulate in the wounded tissue, thus demonstrating that as described in the application, attenuated *S. typhimurim* and *V. cholera* accumulate in immunoprivileged tissues. As discussed in the previous response, and below, Hamblin *et al.* detects infection, not wounded or inflamed tissue. Hamblin *et al.* does not disclose teach or suggest that intravenously administered bacteria accumulate in wounded/inflamed tissue and can be used for imaging/detecting inflamed/wounded tissue. There is no purpose in a method in which bacteria are administered to detect cutaneous wounds – one can examine the subject to find cutaneous wounds. The examples in the application are provided to show that bacteria accumulate in these tissues in a model wound.

As discussed in previous responses, the accumulation of bacteria is **not strain dependent**. The Examiner has cited statements set forth in of the specification to support the argument that the distribution pattern of any particular bacteria is not reasonably predictable. The distribution pattern of any particular bacteria readily can be tested as shown in the application. The cited sections and accompanying working examples, however, show that regardless of any differences in distribution profiles or kinetics, **the tested microorganisms consistently localized at sites of inflammation or wounds** within a subject. **Thus, accumulation is not dependent on the initial distribution.** As claimed, the subject is monitored to determine where bacteria accumulate, not to assess an initial distribution pattern.

The particular sections of the specification that cite differences between the distribution of attenuated *Salmonella typhimurium* and attenuated *Vibrio cholerae* described in Example 2 refer only to the initial observation period only (0-60 minutes) for the experiment; the method demonstrated in the experiment is the monitoring of accumulation. As described in the specification, the experiment involves the injection of attenuated *Salmonella typhimurium* and attenuated *Vibrio cholerae* (each carrying the pLITE201 plasmid for expression of bacterial luciferase) into the left femoral vein of anesthetized mice. Prior to the injection, the left femoral vein was exposed by making a 1 cm incision with a surgical blade. Following injection of the bacteria, the incision was closed with 6-0 sutures, and the mice were then monitored under a low light imager for photon emission. The results

for the initial distribution of the bacterial strains following injection into the mice were shown in Figure 1 of the application and described in Example 2. As stated in Example 2 of the specification:

Injection of attenuated *S. typhimurium* caused wide dissemination of the bacteria throughout the body of the animals (FIG. 1A). This pattern of distribution was visible within 5 minutes after bacterial injection and continued to be detected at the one-hour observation period. Injection of attenuated *V. cholera* into the bloodstream, however, resulted in light emission that was localized to the liver within 5 minutes after bacterial injection and remained visible in the liver at the one-hour observation period (FIG. 1B).

During the initial observation period only, *V. cholera* was seen to localize to the liver and *S. typhimurium* was more widely distributed throughout the animal. One of the primary functions of the liver, widely known at the time of filing of the application, is to clear toxins and foreign materials from the bloodstream. Thus, injected bacteria will accumulate in the liver soon after injection. In view of the knowledge about the liver at the time of the priority date of the application, and the teachings in the specification, one of skill in the art **would not interpret** this initial localization to the liver at five minutes post-injection of the bacteria to indicate that the liver is a site of a wounded or inflamed tissue. The method as described requires monitoring to assess accumulation. Regardless of any reasons why the bacterial strains may have differed in their accumulation in the liver during the initial observation period, this difference had no impact on the results of the experiment, in which both strains exhibited accumulation in the wounded and inflamed tissues after clearance of the initial wave of distribution as shown in Figure 2.

It respectfully is submitted that the statement in Example 2 that “the distribution pattern of light emission following an intravenous injection of bacteria into the mice was bacterial-strain-dependent” should not be taken out of context of the experiment as a whole. The statement refers only to the initial observation period of the experiment and has no bearing on the outcome, namely, both bacterial strains accumulated in the wounded/inflamed tissues and not in uninjured tissues. This clearly is shown in Figure 2 as well as stated in specification:

Imaging the same animals 48 h after bacterial injection showed that **all** of the detectable light emission from the earlier time had diminished and was eliminated completely from the injected animal with the exception of the inflamed wounded tissues such as the incision wound and the ear tag region...Careful examination of individually excised organs as well as blood samples from infected animals confirmed the absence of luminescence in these normal uninjured tissues.”[emphasis added].

Hence, the Examiner’s assertion regarding the difference in the distribution among the bacterial strains has no bearing on the ability of both tested strains to accumulate in

wound/inflamed tissues, as supported by the Examples. Furthermore, with reference the data shown in the Figures, *it is emphasized that the leg wound in Figure 4 would not be visible from the dorsal view shown, which allows observation of the ear tag wound.* Also, it is noted that bacteria do not accumulate at sites that are not inflamed or have healed. Hence, the bacteria will not accumulate at an ear tag that is healed or not inflamed. The application shows that bacteria accumulate in wounded/inflamed tissues/sites and are cleared from healthy tissues. **Thus, differences in initial distribution of bacteria has no bearing on practice of the claimed methods.**

c. The Examiner continues:

The application further teaches that when inflammation was induced at a rat aortic valve by implanting a catheter near the heart valve, intravenously injected light emitting *E. coli* were found to accumulate in the heart of catheterized animals but not control animals. (Example 3 and Figure 6.)

It is noted that the application presents no data at all with respect to bacteria other than those strains listed above. With regard to the findings presented, the specification teaches, "In the experiments leading to the present invention it has been found that inflamed tissues, e.g. near implanted material, permit bacterial colonization." (Paragraph bridging pages 3-4.)

As discussed above, the application teaches that wounded/inflamed tissues are immunoprivileged so that bacteria that possess the requisite properties: replicate in the subject; not pathogenic to the subject and is recognized by the immune system of the subject accumulate in wounded/inflamed tissues when administered systemically. The specification demonstrates this with three exemplary diverse species of bacteria. There is no requirement in patent law that all species within the scope of a claim must be exemplified.

As stated above, the application teaches that wounded/inflamed tissue are immunoprivileged and that the bacteria that are intravenously administered that meet the three requirements accumulate in immunoprivileged tissues. The Working examples that use cutaneous wounds do so as a model system to demonstrate that the intravenously administered bacteria accumulate in the wounds, thus demonstrating that systemically administered bacteria that exhibit the requisite properties are cleared from all other tissues, but accumulate in wounded/inflamed tissues. The third example, shows that bacteria accumulate in internal wounds. All of the examples together demonstrate that bacteria that replicate in the subject; not pathogenic to the subject and is recognized by the immune system of the subject accumulate in wounded/inflamed tissues when administered systemically and that by virtue of their accumulation, bacteria that are detectable, permit detection of wounded/inflamed tissues.

d. The Examiner continues:

The application then goes on to assert that the finding that tissues that are irritated by implanted materials are more susceptible to bacterial colonization "open the way for (a) designing multifunctional viral vectors useful for the detection of wounded or inflamed tissue based on signals like light emission or signals that can be visualized by MRI and (b) the development of bacterium- and mammalian cell-based wounded or inflamed tissue targeting systems..." (Paragraph bridging pages 4-5; emphasis added.)

Thus, the application demonstrates that some strains of bacteria will colonize areas in which foreign bodies have been implanted and seeks to claim using any bacterium having the capacity to accumulate at the location of any wounded or inflamed tissue inside a subject with sufficient specificity that the detectable accumulation of the bacterium can be used to indicate the location of wounded or inflamed tissue. At the same time, the application acknowledges that what is demonstrated merely "opens the way for" designing vectors useful for detection of wounded or inflamed tissue and the development of bacterium-based wounded or inflamed tissue targeting systems.

Applicant respectfully disagrees with the Examiner's inference, that this turn of phrase means that the application does not teach vectors that can be used to express detectable proteins in bacteria. This is clearly not what is meant nor shown in the application. The application describes vectors and their use and detectable proteins in great detail (see, *e.g.*, page 7, paragraph 2, - page 11, paragraph 3, page 12, first full paragraph, and the working examples, which employ vectors, such as pLITE201, that express detectable proteins. Those of skill in the art are well-acquainted with detectable proteins and proteins that induce a detectable signal, as well as bacteria, that by virtue of accumulation of a product, such as a heavy metal, are detectable, such as by light emission or PET imaging as described in the application.

e. The Examiner continues:

With regard to accumulation of any bacterium at sites of inflammation other than wounds or tissue into which a foreign object has been introduced, *e.g.*, an atherosclerotic lesion, the application refers to reports providing evidence that *C. Pneumonia*, *H pylori*, CMV and HSV have been found in atherosclerotic plaques and speculates that intravenously administered microorganisms will penetrate into atherosclerotic plaques where they will replicate to a sufficient degree that they will be capable of indicating the presence of a plaque. (See especially the paragraph bridging pages 13-14.) However, no evidence is presented to indicate that any intravenously administered bacterium would be capable of selective accumulation within an atherosclerotic lesion such that it could actually be used to identify the location of the lesion as claimed.

It respectfully is submitted that there is no requirement in the claims nor by virtue of the method for "selective" accumulation. As described in the application and discussed above, the application teaches that wounded/inflamed tissues are immunoprivileged and teaches how to exploit this. The application shows that systemically administered bacteria that possess the

requisite properties are cleared by the subject's immune system, except in immunoprivileged tissues, and that wounds/inflamed tissues are immunoprivileged. Thus, bacteria with the requisite properties will accumulate in such tissues. Atherosclerotic plaques contain inflamed tissues; thus, as described in the application, bacteria will accumulate in such plaques (as well as any other wounded/inflamed tissues the subject has. One of skill in the art will infer whether or not a site of accumulation could be an atherosclerotic plaque by virtue of its locus and factors. The instantly claimed method is for visualizing wounded/inflamed tissues *per se*. Such wounded/inflamed tissues can be associated with particular conditions.

f. The Examiner continues

State of the prior art and level of predictability in the art: The "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. If one skilled in the art can readily anticipate the effect of a change within the subject matter to which the claimed invention pertains, then there is predictability in the art. On the other hand, if one skilled in the art cannot readily anticipate the effect of a change within the subject matter to which that claimed invention pertains, then there is lack of predictability in the art. Accordingly, what is known in the art provides evidence as to the question of predictability.

The physiological art is recognized as unpredictable. (MPEP 2164.03.) In cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws. In cases involving unpredictable factors, such as most chemical reactions and physiological activity, the scope of enablement obviously varies inversely with the degree of unpredictability of the factors involved.

Applicant respectfully disagrees. Predictability can be shown by reproducibility and an understanding of the basis for the results achieved. The instant application teaches and shows, with several diverse species, that bacteria with the requisite properties, when administered systemically, accumulate in immunoprivileged tissues, and shows and teaches that wounded/inflamed tissues are immunoprivileged. Therefore, the results are reproducible and predictable.

g. The Examiner continues:

In addition to the general unpredictability of the physiological arts, the unpredictability of the art related to the instant invention is clearly evidenced by the teachings of the instant application. In the paragraph bridging pages 1-2, the specification teaches (emphasis added):

Bacteremias may arise from traumatic injuries and surgical procedures as well as from physiological functions...A potential consequence of bacteremia is colonization of susceptible sites. However, despite the occurrence of transient bacteremias, only a certain percentage of high-risk patients develop bacterial colonization of potentially susceptible sites. A number of investigators have suggested that bacteria from the blood circulation can colonize inflamed tissues in animal models and on the surface of implanted materials. The inconsistency in

the pathological changes in humans following a bacteremia may also be due to the resistance of host immune system, the variability in the concentration of bacteria in the blood subsequent to different bacteremia events, and the virulence of any given bacterial strain.

Thus, the application teaches that the colonization of potentially susceptible sites by any given bacterium is variable and might be dependent on the properties of the host, the amount of bacteria present and the properties of the bacterial strain. This variability is also evidenced by the working examples, which show that different wounds were colonized depending upon the bacterial strain injected and the mouse strain used in the experiment.

Applicant respectfully disagrees. As discussed above, this is not correct. Attention is directed to the discussion above, which begins:

As discussed in previous responses, the accumulation of bacteria is not strain dependent. The Examiner has cited statements set forth in of the specification to support the argument that the distribution pattern of any particular bacteria is not reasonably predictable. The distribution pattern of any particular bacteria readily can be tested as shown in the application. The cited sections and accompanying working examples, however, show that regardless of any differences in distribution profiles or kinetics, the tested microorganisms consistently localized at sites of inflammation or wounds within a subject. Thus, accumulation is not dependent on the initial distribution. As claimed, the subject is monitored to determine where bacteria accumulate, not to assess an initial distribution pattern. . . .

. . . Hence, the Examiner's assertion regarding the difference in the distribution among the bacterial strains has no bearing on the ability of both tested strains to accumulate in wound/inflamed tissues, as supported by the Examples. Furthermore, with reference the data shown in the Figures, it is emphasized that the leg wound in Figure 4 would not be visible from the dorsal view shown, which allows observation of the ear tag wound. Also, it is noted that bacteria do not accumulate at sites that are not inflamed or have healed. Hence, the bacteria will not accumulate at an ear tag that is healed or not inflamed. The application shows that bacteria accumulate in wounded/inflamed tissues/sites and are cleared from healthy tissues. Thus, differences in initial distribution of bacteria has no bearing on practice of the claimed methods.

The results shown in the application with the different species of bacteria are consistent. One cannot look at the initial distribution, but the accumulation of the bacteria following clearance by the immune system. As amended, the claims capture this by explicitly reciting: "after a sufficient time for the bacterium to accumulate in wounded or inflamed tissues inside of the subject, imaging or monitoring the detectable bacterium to detect accumulation of the bacterium in the subject, thereby detecting or imaging the wounded or inflamed tissues inside of the subject."

h. The Examiner then refers to Yu *et al.* (2003) Anal. Bioanal. Chem. 377: 964-72 (of record). Yu *et al.*, as discussed in previous responses, and repeated below, describes that tumors and tumor tissues are immunoprivileged tissues and shows, using tumor models, not wound/inflammatory models, that all of these bacteria and (viruses with the requisite

properties) accumulate in tumors. Since the model has tumors, not wounds, there is not going to accumulation in wounds or inflamed tissues.

Examiner maintains that Yu *et al.* (2003) demonstrates Yu *et al.* demonstrates that bacteria, viruses or mammalian cells, when administered to subjects, accumulate in cancerous tissues, but not in any tissue recited in the claimed subject matter. The Examiner further alleges that Yu *et al.* teaches that the mechanism of bacterial colonization is unknown, that administration type-dependent colonization is common and that it is not “predictable” which administration will yield which colonization type.

First, Yu *et al.*, employs tumor models, not inflammation/wound models, and shows that bacteria accumulate in tumorous tissue. Yu *et al.* does not show anything regarding wounded or inflamed tissues/sites. Yu *et al.*, which published after the earliest priority date of the instant application date, describes methods for detection of tumors. Hence, it is not pertinent to the methods of the instant claims. Therefore, the Examiner’s statement that Yu *et al.* is not enabling for the instantly claimed subject matter, is inapt, since Yu *et al.* is directed to different subject matter and is published subsequent to the priority date of the instant application. Yu *et al.*, is irrelevant to whether or not the application teaches how to make and/or use the claimed method, which is for detecting wounds/inflamed tissues.

Second, knowledge of the mechanism of colonization or colonization-type is irrelevant to the practice of the methods as claimed. By following the teachings of the specification, the bacteria are administered, and, as taught and demonstrated in the application, bacteria, with the recited properties, accumulate at wounded/inflamed tissues/sites. The teachings of the specification, in light of the state of the art and the knowledge of those of skill in the art, allow one of skill in the art to practice the steps of the methods as claimed. Knowledge of the mechanism of localization or the types of colonies visualized at the sites of localization, is not needed. The specification teaches and demonstrates that bacteria that are recognized by the immune system and that are nonpathogenic or attenuated accumulate at such sites/tissues.

As discussed above, to demonstrate the full scope of enablement of the claimed methods, Applicant is not required to demonstrate a completely optimized procedure as long as it is possible to successfully perform the method as claimed without undue experimentation. Contrary to the assertion of the Office Action, the ability to practice the methods as claimed is not dependent on the particular bacterium that is used in the method. Instead the non-pathogenic or attenuated microorganism or cell is a tool that is used to detect abnormal conditions in a subject, such a wound or inflamed tissue, a foreign object (*e.g.*, a

suture), or a disease or condition associated with wounded or inflamed tissue. It is not a characteristic of the particular microorganism or cell per se that provides for specific colonization of the wounded or inflamed tissue, but rather is the protective environment of the wounded or inflamed tissue (from the immune system) that leads to accumulation of bacteria in such sites. The bacteria administered are cleared by the immune system from non-wounded or non-inflamed tissues. Hence, any non-pathogenic or attenuated microorganism or cell that is recognized by the immune system should accumulate in wounded or inflamed tissue as taught by the specification. The claimed methods are thus applicable to any non-pathogenic or attenuated microorganism or cell that is recognized by the immune system. Such microorganisms and cells will be cleared from most tissues/sites, but not from wounded/inflamed tissues/sites.

i. Rebuttal Examiner's argument that the claims are not enabled for detecting an atherosclerotic plaque

It respectfully is submitted that the application demonstrates that bacteria as claimed accumulate in wounded tissues and inflamed tissues within a subject. Those of skill in the art know that atherosclerotic plaques are inflamed lesions and their locus is known.

Atherosclerosis is a chronic inflammatory response in the arteries. Bacteria, with the requisite properties (recognized by the immune system and replicate in the subject) will, upon administration, will be recognized by the immune system and cleared from non-wounded, non-inflamed tissues, but will, by virtue of the immunoprivileged nature of wounded and inflamed tissues, will not be cleared from such tissues and will accumulate and will be detected. Thus, detection of accumulated bacteria in the arteries is indicative of an atherosclerotic plaque.

j. Contrary to the assertion of the Examiner, the instant application teaches, in view of the breadth of the claims, the level of skill and knowledge of the skilled artisan, and the shown reproducibility of the method, including the working examples, that a bacteria that replicate in the subject; not pathogenic to the subject and is recognized by the immune system of the subject accumulate in wounded/inflamed tissues when administered systemically, accumulate in immunoprivileged tissues and that wounded/inflamed tissue are immunoprivileged. In view of this information and the required properties of the bacteria (as claimed), one of skill in the art readily can select a bacterium that is detectable, administer it to a subject and detect inflamed/wounded tissue within the subject without undue experimentation.

As discussed above, and shown in the application, bacteria for use in the methods are known, and the methods for detection of the bacteria are known. As discussed above, the bacteria and means for detection/visualization are known reagents that can be used in the instantly claimed methods. The application teaches that bacteria accumulate in wounds/inflamed tissues/sites and demonstrates that with working examples using three different strains. There is no basis for the Examiner to conclude that other strains known to have the properties recited in the claims do not accumulate in wounds/inflamed tissues/sites. Further, if needed, one of skill in the art can test a candidate strain that meets the recited criteria exactly as taught in the specification to confirm that the strain accumulates. No knowledge of the mechanism for accumulation is needed. Further as described above, detection/visualization methods are known to those of skill in the art and also are described in the application. One of skill in the art can employ any such method.

Policy Considerations

As demonstrated by the above of the *In re Wands* factors, the teachings of the specification, when combined with the knowledge of those of skill in the art and the ability to repeatedly and successfully (i.e., predictably) execute the various steps, leads to the conclusion that each of the steps of the instant methods could be performed without undue experimentation. As discussed above, administration of microorganisms for the detection of wounded or inflamed tissue was successfully demonstrated using a variety of microorganisms by following the teachings of the instant application and by an extensive body of knowledge in the art as of the application's earliest priority date.

Applicant is entitled to claims that are commensurate in scope, not only with what Applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the Applicant has disclosed. In the instant application, Applicant provides the public with methods for the detection of a wound, wounded tissue, an inflammation site, inflamed tissue thereof by administering and detecting a variety of microorganisms or cells that accumulate at such sites. As a broad body of knowledge is available in the areas of microbiology, genetic manipulation of microorganisms and cells, administration of microorganisms and cells to subjects, and *in vivo* detection techniques for detecting the administered microorganisms and cells, and the application teaches bacteria for use in the method and how to identify such bacteria, and provides working examples with three diverse species, it would be unfair and contrary to the Constitutional mandate set forth in Article, Section 8, to require applicant to limit the claims to any particular embodiment or to deny patent protection at all. To do so would permit those

of skill in the art to practice the methods as described in the application with any bacterium that has the requisite properties using known detection/visualization method and avoid infringing such limited claims.

See, e.g., In re Goffe, 542 F.2d 801, 166 USPQ 85 (CCPA 1970).

The public purpose on which the patent law rests requires the granting of claims commensurate in scope with the invention disclosed. This requires as much the granting of broad claims on broad inventions as it does the granting of more specific claims on more specific inventions." *In re Sus and Schafer*, 49 CCPA 1301, 306 F.2d 494, 134 USPQ 301, at 304.

THE REJECTION OF CLAIMS 1, 2, 9, 12, 18 AND 22 UNDER 35 U.S.C. §102(b)

Rejection of Claims 1, 2, 9, 12, 18 and 22 under 35 U.S.C. §102(b)

Claims 1, 2, 9, 12, 18 and 22 are rejected under 35 USC §102(b) as anticipated by *Fu et al.* because *Fu et al.* allegedly discloses a method for detecting wounded or inflamed tissue inside of a subject, comprising systemically administering to a subject a detectable, non-pathogenic bacterium that replicates in the subject, is recognized by the immune system of the subject and is not targeted; and monitoring the subject to detect the accumulation of the bacterium at or in a wounded tissue or inflamed tissue inside of the subject. The Examiner stated in the previous Office Action that the *E. coli* was recognized and cleared by the immune system, and thus is considered nonpathogenic, and uses the pUC19 plasmid, which encodes the antibiotic ampicillin as a therapeutic agent. The Examiner alleges that because "living bacteria were found to have reached the burn tissue after traveling through the stomach, lining of the gut, and the liver, they replicate in the subject". The Examiner continues and states that because ampicillin was expressed, the *E. coli* are considered to comprise an inducible promoter regulating expression of ampicillin, that is, expression of ampicillin from the pUC19 vector is inducible upon introduction of the vector into a bacterial cell. This rejection respectfully is traversed.

Relevant Law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Spada*, 15 USPQ2d 1655 (Fed. Cir, 1990), *In re Bond*, 15 USPQ 1566 (Fed. Cir. 1990), *Soundsciber Corp. v. U.S.*, 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." *In re Lang*, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). It is incumbent on Examiner to identify wherein each and every facet of the claimed invention is

disclosed in the reference. *Lindemann Maschinen-fabrik GmbH v. American Hoist and Derrick Co.*, 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference. *In re Oelrich*, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

“Rejections under 35 U.S.C. §102 are proper only when the claimed subject matter is identically disclosed or described in the 'prior art' . . . the [r]eference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without *any* need for picking, choosing, and combining various disclosures not directly related to each other by the teachings in the cited references. Such picking and choosing may be entirely proper when making a rejection of a §103, obviousness rejection, where the applicant must be afforded an opportunity to rebut with objective evidence any inference of obviousness which may arise from the *similarity* of the subject matter which he claims to the prior art, but it has no place in the making of a §102, anticipation rejection.” (Emphasis in original). *In re Arkey, Eardly, and Long*, 455 F.2d 586, 172 USPQ 524 (CCPA, 1972).

The Claims

The claims are discussed above.

Disclosure of *Fu et al.* and differences from the instant claims

Fu et al. is directed to a study of the role of bacteria in the gut in severe burn infections. ***Fu et al.*, does not disclose any method for detection of wounds or inflamed tissues nor any step of detecting wounded and inflamed tissues.** *Fu et al.* is detecting bacteria in order to study trafficking of intestinal bacteria in infections following severe burns. *Fu et al.* concludes that intestinal bacteria can traffic through the damaged intestinal wall and call infections.

Fu et al. provides a study assessing the role that fecal organisms play in burn wound infection. To study this role, *Fu et al.* provides an animal model to observe the:

dynamics of fecal organisms and burn wound organisms in attempts to investigate the relationship between translocation infection by fecal organisms and burn wound infection more precisely.

Thus, *Fu et al.* does not disclose or even hint at a method for detecting wounds or inflamed tissues.

To assess the role of fecal organism translocation in infecting burn wound, fluorescence labeled bacteria were introduced into Wistar rats through a gastric catheter, **followed by third degree burning.** Thus, the bacteria **are not introduced into** a subject in whom wounded/inflamed tissues are to be detected, but into a subject that is to be **burned.**

After predetermined time periods, the rats were sacrificed and organs excised to assess bacterial infection by fluorescence. In another group of rats, bacteria containing pUC19 to provide ampicillin resistance for selection (not therapy) were inoculated into the intestines of the rats through a catheter, and fed ampicillin to select for the introduced bacteria. After confirming that the Amp-resistant bacteria were incorporated into the intestinal tract, the rats were burned, and then sacrificed and their organs harvested and bacteria cultured in medium containing ampicillin. Plasmid was extracted and identified by restriction digestion patterns.

Fu *et al.* states that the results indicate that in the early stage of a severe burn, intestinal bacteria can penetrate through the damaged intestinal membranous barrier and disperse. Thus, enterogenous infection should be considered in cases of sepsis in early burn stages.

Fu *et al.*, thus, discloses that intestinal flora can cause sepsis in severe burns. Fu *et al.* provides an animal model for studying such infections. Fu *et al.* does not disclose a method for detection of wound and inflamed tissues; Fu *et al.* is studying infection following severe burns; no detection of wounds and inflamed tissue is involved. Fu *et al.* does not disclose systemically administering detectable bacteria to a subject, and then detecting accumulation of the bacteria in order to identify the location of a wound or inflamed tissue inside of the subject. While Fu *et al.* uses detectable bacteria, the method is for detecting bacteria, by administering bacteria and burning a subject to see where the bacteria traffic in the subject. This is not a method for detecting wounds or inflamed tissue in a subject by administering bacteria. Fu *et al.*, thus, does not disclose all elements of claim 1 nor any claim. Therefore, Fu *et al.* does not anticipate any pending claim.

Rebuttal to comments of the Examiner:

The Examiner states:

detecting bacteria at or in a wounded or inflamed tissue is an active method step recited in claim 1. Applicant's assertion is also a selective reading of Fu *et al.*, as the detection of the labeled *E. coli* within the burn wounds is found throughout Fu *et al.* (i.e. the subeschar tissue analyzed by Fu *et al.* is by definition "inside" a subject: it is underneath the dead or necrotic tissue caused by the burn). This is all that is required to anticipate the broadly-worded method steps of "monitoring...the accumulation of the bacterium at or in a wounded tissue or inflamed tissue...wherein detection of accumulation indicates the location of wounded or inflamed tissue." The last step (i.e. "detection...indicates the location") is apparently a mental process performed after detection of the bacterium, which is precisely the association Fu *et al.* make regarding the location of the labeled bacteria with the burn wounds.

Applicant respectfully disagree. The instant method is for detecting wounded/inflamed tissue inside of a subject. Fu *et al.* is studying trafficking of bacteria in a subject with b administering the bacteria and then burning the subject to assess the role of fecal bacteria in causing sepsis. Thus, Fu *et al.* does not describe a method for imaging or detecting wounded/inflamed tissue inside of a subject who is suspected of having a wound or burn. Fu *et al.* does no disclose administering the bacteria to “a subject in whom the presence or absence of a wounded tissue or inflamed tissue or a disease associated therewith is to be detected.” Fu *et al.* administers the bacteria to a subject, causing a burn and then looking to see where the bacteria traffic. Thus, Fu *et al.* does not disclose the step of systemically administering a bacterium for detecting wounded or inflamed tissue. The bacteria are administered for infecting a subject assess sepsis and the method includes a step of burning a subject.

Fu *et al.*, which studies the trafficking of bacteria to burns (administering bacteria and then burning a subject), does not disclose a step of “identifying a subject to be tested for the presence or absence of wounded tissue or inflamed tissue therein.” Its study is for studying bacterial trafficking, not wounds, and its does not “identifying a subject to be tested for the presence or absence of wounded tissue or inflamed tissue therein,” since it is not testing for identification of wounded or inflamed tissue.

The bacteria are introduced into the gut into Wistar rats **through a gastric catheter directly into the gut, not systemically (which involves piercing of skin or mucous membranes or veins or injection, such as IV, or IM, or orally into the digestive system)**. Thus, Fu *et al.* does not disclose systemic administration of a bacterium.

Fu *et al.* does not disclose a step of “after a sufficient time for the bacterium to accumulate in wounded or inflamed tissues inside of the subject, imaging the detectable bacterium inside of the subject to detect accumulation of the bacterium in the subject, and identifying wounded or inflamed tissues inside of the subject.” This is more than a mental step. The subject in whom wounded/inflamed tissue is to be assessed has the wounded or inflamed tissue is identified. This is an affirmative step. Fu *et al.* does not disclose a method for identifying wounded or inflamed tissue. The method of Fu *et al.* includes a step of **burning of animals**. The instant method does not, inherently, or otherwise, include a step of inflicting a wound or causing inflammation of a subject. Furthermore, in the method of Fu *et al.*, the bacteria are administered, and **then** the animals are burned. In the instantly claimed method, the subject is one in whom a wounded or inflamed tissue is to be detected. The

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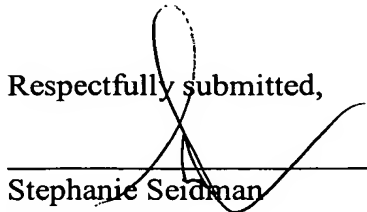
Attorney Docket No.: 3800002.00055/4804US
Amendment and Response

subject must already have the wound or inflamed tissue; it is not created after administering the bacteria.

* * *

In view of the above, reconsideration and allowance of the application respectfully are requested.

Respectfully submitted,



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Reg. No. 33,779

Attorney Docket No. 3800002.00055/4804US
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